



Visualising SYBR Green™ Using a Syngene Image Capture System

What is SYBR Green™?

SYBR Green™ from Invitrogen (Molecular Probes) is increasingly used as an alternative to Ethidium bromide in the visualisation of nucleic acids. SYBR Green chelates (binds) directly to the nucleic acid. Upon excitation with an appropriate light source, a green light is emitted that can be imaged and quantified.

There are two main reasons for the use of SYBR Green in the laboratory. The first is that it is less mutagenic than ethidium bromide. However, users often over estimate its safety. Although less mutagenic, SYBR Green is still a very dangerous chemical that should be handled with extreme care. As SYBR Green is considerably more expensive than ethidium bromide, the main reason it is used is for its much enhanced sensitivity. There are two types of SYBR Green; SYBR Green I preferentially stains dsDNA. SYBR Green I is greater than 25-fold more sensitive than ethidium bromide in the detection of nucleic acids. SYBR Green II shows good sensitivity for RNA, while also staining dsDNA and ssDNA. The visualisation procedures are different for both the stains.

Visualisation

SYBR Green I and II can be visualised extremely well using all Syngene image capture systems. There are a number of configurations that can be used, and the user should experiment and determine which works best for them.

SYBR Green I has three excitation peaks, 290, 380 and 497nm and as such mid UV (302nm) long UV (365nm) and blue light, respectively, can be used for excitation. For UV illumination best results will be achieved by using the short pass filter. When utilising the Syngene blue light converter a SG emission filter should be purchased. In this case the short pass filter cannot be used as there will be an overlap between the excitation and emission spectra. Please consult Syngene for details of the SG emission filter.

SYBR Green II has two excitation peaks, 254 and 497nm. The most sensitive option, as advised by Invitrogen (Molecular Probes), is to use epi UV at 254 nm. This option can be fitted to all Syngene darkrooms. This method will give excellent band definition and low background. However, integration times will be significantly longer than those used for ethidium bromide gels, sometimes up to 2-3 seconds. Trans UV may be used at 302nm (standard). Background may be a little higher, but band definition will be good, and integration times shorter, but still longer than for conventional ethidium bromide. The third illumination option is that of blue light by utilising the Syngene blue light converter screen. Although less sensitive than UV, some users prefer this option from a safety point of view – the gel may be viewed by eye without the use of safety precautions. As with SYBR Green I a SG emission filter should be selected.

Filters may be screwed directly onto the camera in the DigiGenius system. In the darkroom based systems, motorised filter wheel and filter slider options should be utilised. In the GeneFlash and InGenius the filters may be interchanged in the filter drawer.

